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Role of malic acid in enhancing the efficiency of barley (Hordeum vulgare L.) and alfalfa (Medicago sativa L.) plants for phytoremediation of salt affected soil

Sara F. Radhi, Luma H. Abdul-Qadir[*]

Department of Biology, College of Education for Pure Sciences, University of Basrah, Basrah,Iraq.

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ABSTRACT

Soil salinization is a growing global problem that influences plant growth and crop productivity. Most of the reclamation efforts in the past have focused on the installation of surface drainage systems. Other management approaches, such as excessive leaching and chemical amendments, have also been used on a limited scale to enhance the productivity of these soils. Phytoremediation can be cost-effective and environmentally sound technology. A laboratory experiment was carried out to study the role of malic acid which is low molecular weight organic acid (LMWOA) in enhancing the efficiency of barley and alfalfa plants for the phytoremediation of salt-affected soil. Seeds of barley and alfalfa were cultured in pots and irrigated with full strength Hoagland nutrient solution with three concentrations of seawater (SW) (10%, 20% and 30%) and a mixture of seawater with malic acid (MA) at 2, 4 and 6 mM l^{-1} (MA+SW), Hoagland solution was used as control. After twelve weeks, plants were harvested, and three types of soils (barley soil, alfalfa soil, and plant-free soil) were subjected to physical and chemical analysis for EC (electrical conductivity), TOC (total organic carbon), pH, potassium, sodium, and chloride ions. Results indicated a significant decrease was recorded in soil EC, pH, potassium, sodium, and chloride ions and a significant increase in soil TOC in barley and alfalfa soil compared with plant-free soil. Treatments with (MA+SW), especially at (2+10%) resulted in a significant increase in ions availability and phytoremediation activity in barley and alfalfa soils comparing with plant-free soil.

1. Introduction

Soil salinization is a growing global problem that influences plant growth and crop productivity [1]. Salinity stress negatively affects plant cells photosynthesis, respiration, and protein synthesis

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[*] **Corresponding author email :** luma.abdulqadir@uobasrah.edu.iq

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[2]. Approximately 1.1x 10^9 ha of land worldwide is salt affected, and this number is still increasing by 1.5 million hectares (mha) per year [3].

In Iraq, the total geographic area is 45mha, out of which 34 mha (78%) is unsuitable for agriculture under current conditions [4]. According to FAO estimates, the total cultivated area of Iraq is 6 mha, out of which 50 percent in northern Iraq is in rain-fed condition while the rest is irrigated [5]. Most of the past reclamation efforts in the past have focused on the installation of surface drainage systems; other management approaches such as excessive leaching and chemical amendments, have also been used on a limited scale to enhance the productivity of these soils [4]. However, success has been limited, and the problems of salinity kept increasing [6]. These operations are costly and labor-intensive for the continuous and global issue of salinity, and there are various ways to remediate saline soils; among them is phytoremediation can be cost-effective and environmentally sound technology [7].

Phytoremediation, also known as vegetative bioremediation, is an approach for saline soil remediation by cultivating of salt-accumulating or salt-tolerant plants and is perceived as a sustainable and cost-effective technique. The plant species used for phytoremediation are mainly halophyte, hyperaccumulator, salt-tolerant, or transgenic plants [7]. LMWOA, such as malic, citric, and acetic acids can bind metals forming complexes and changing their bioavailability [8]; [9]. Using these easily biodegradable chelates has been proposed to enhance the metal soil availability and accumulation in plants while avoiding leaching risks [10]. In recent years, the application of LMWOA has proven successful in reducing heavy toxicities in soils [11]. Malic acid ($C_4H_6O_5$) which is synthesized by plants and microbes, is less detrimental to the environment and plants than synthetic chelates since it is biodegradable, forming strong ligands with metals and increasing resistance to toxic elements. [12]; [13].

Alfalfa (*Medicago sativa* L.) is a moderately saline-tolerant legume [14]. It is an important forage source for livestock industries around the world because of its wide adaptability, high yield, good quality, and resistance to frequent cuttings [15]. Barley (*Hordeum vulgare* L.) is an important salt-tolerant cereal crop among the world's earliest domesticated crop plants [16]. Both crops were cultivated in Iraq for use as animal feed. So, the present study aimed to evaluate the role of malic acid in enhancing the ability of barley and alfalfa plants for the phytoremediation of saline soil.

2. Materials and Methods

2.1. Experiment site

A laboratory experiment was conducted at the plant physiology & tissue culture laboratory, college of Education for pure sciences, University of Basrah, to evaluate the role of malic acid in enhancing the efficiency of barley (*Hordeum vulgare* cv. Sameer) and alfalfa (*Medicago sativa* local cultivar) plants for the phytoremediation of salt affected soil.

2.2. Soil preparation and plant culturing

The soil was brought from a local farm then well mixed and air dried and subjected to physical and chemical analysis table 1. to determine the soil texture [17], pH and electrical conductivity [18], total organic carbon [19], organic matter [20], sodium, and chloride ions [18].

2.3. Treatments

Three concentrations of seawater (SW) (10% 20% and 30%) were added to full strength Hoagland nutrient solution. A combination of seawater at previous concentrations with three concentrations (2-, 4-, and 6-mM l^{-1}) of malic acid i.e. (MA+SW), in addition to control treatment with the Hoagland nutrients solution only, was used to irrigate pots filled with soil according to field capacity [18].

2.4.Seeds cultivation

Seeds were surface sterilized by ethanol 70% for 5 minutes and then rinsed with distilled water many times. Two and a half kilograms of the blended soils were put into pots, barley and alfalfa seeds were planted in each pot, and plant-free soil was used as a control for comparing the role of malic acid in enhancing the phytoremediation efficiency of each plant. Ten seeds of each plant were planted in pots for fourteen days and irrigated with Hoagland nutrient solution only, then thinned to five at the early seedling stage, three replicates for each treatment were made. Treatments with SW and (MA+SW) were started at the early seedling stage and continued for twelve weeks at the growth room (temperature 25±2 ̊C), (16/8 light/dark period with white fluorescence light). Plants harvested after twelve weeks and three types of soil samples (alfalfa soil, barley soil, and plant-free soil) were collected and analyzed for the estimation of electrical conductivity (EC), pH, total organic compounds (TOC), potassium, sodium, and chloride ions.

Table 1. Soil physical and chemical characters

Texture: Sandy Clay Loam	Silt 23.98% Clay 26.24% Sand 49.78%
EC (ds m^{-1}) 1:1	2.22
pH 1:1	9.8
Chloride (mg g^{-1})	0.29
Sodium (mg g^{-1})	11.98
Total nitrogen (mg g^{-1})	15.7
Total organic carbon (TOC) (mg g^{-1})	3.36
Organic matter (mg g^{-1})	9.11

2.5. Statistical analysis

The experiment was arranged in a completely randomized design (CRD) for a factorial experiment with two factors (3 soil types X 13 treatments) and three replicates for each treatment were made. The results were subjected to a two-way analysis of variance (ANOVA) using SPSS V.23. After getting ANOVA table, the means were compared by a revised least significant difference test (RLSD) at a probability of 0.05.

3. Results and discussion

3.1 Electrical conductivity (EC)

Table 2. results indicate that increasing sea water concentration leads to a significant increase in EC at all treatments compared with the control. The higher level which was 17.60 ds m^{-1} recorded at combination of 30% seawater with 6 mM malic acid. Soil EC results showed that barley soil achieved the lowest degree of electrical conductivity (6.90 ds.m^{-1}), which was significantly less than alfalfa soil (7.35 ds.m^{-1}) and plant-free soil (16.38 ds.m^{-1}). Regarding interactions between salinity and soil type, the results showed that the EC of plant free-soil at 30% seawater was 18.70 ds.m^{-1} while it was 11.33 and 12.57 for barley and alfalfa soils, respectively, which means that barley and alfalfa plant absorbed 39.5% and 32.8% of the soluble salts respectively. EC for a combination of 30% seawater with 2 mM malic acid was 24.54 ds m^{-1} while it was 6.84 ds m^{-1} and 5.27 ds m^{-1} for barely and alfalfa soil, which means that this combination caused the highest levels of soil soluble salts remediation through absorption which was 72.2% in barley and 78.6 for alfalfa.

3.2. Soil total organic carbon (TOC)

Table 3. show that increasing salinity level caused a significant increase in TOC at the combination of malic acid with seawater at 2+10, 2+20, and 2+30, while there was a significant

decrease in other treatments compared with control and 10% seawater treatments. Alfalfa plant soil TOC (3.98 %) was significantly superior to that of barley soil (3.42%) and plant-free soil (2.31%). Interactions result showed that highest levels of TOC recorded at interaction of alfalfa soil with 2+10 (4.76%) which was significantly higher than other interactions.

3.3. Soil pH

Table 4. indicates that increasing seawater concentration caused significant increase in soil pH as an average of all soil types, reaching the highest level at 20% SW with 9.51 compared with control (8.85). All treatments of MA+SW caused significant decreases in pH except at 6+30 with 9.03. Alfalfa plant soil pH of 8.67 was significantly less than that of barley plant and plant-free soil. Interaction results showed that SW+MA at 2+30 with alfalfa soil has the lowest pH with pH with 7.67.

3.4. Soil potassium, sodium, and chloride ions

Treatments with SW and MA+SW caused significant increases in potassium ion table 5. and sodium ion concentrations table 6. compared with control treatment; the higher concentrations recorded were at treatment 6+20 and 6x30 for potassium and 6+30 for sodium. Plant-free soil accumulates a high concentration of potassium (4.07) which exceed significantly that accumulates in barley and alfalfa soils (0.84 and 0.85), respectively it also accumulates a high concentration of sodium (34.81) compared with 14.84 for barley and 15.74 for alfalfa soil. Interaction results between MA+SA with alfalfa and barley soils show significant decrease in potassium and sodium ions concentrations compared with control, with no significant differences between the two soils.

Regarding chloride ion table 7. the same manner was recorded as sodium and potassium i.e., plant-free soil accumulates the higher chloride concentration (188.27), which significantly exceeds barley soil (120.43), and alfalfa soil (116.38). Table 6. shows also that increasing SW concentration and the complications of MA+SW caused a significant increase in chloride concentration compared with the control. The interaction results indicate that barley and alfalfa soils have lower concentrations of chloride, which is significantly different compared with plant-free soil at all interactions treatments.

Results of the study indicated that EC, pH, TOC, and different soil ionic content like potassium, sodium, and chloride declines in barley and alfalfa soils after plantation and harvesting the plants, the same results reported by [21]. like many other plants it seems that barley and alfalfa plants uptake salt ions from soil and store them in high concentrations in their shoots. Accumulation may occur in roots also which is an efficient salt management mechanism [22]. The study also indicated that malic acid, which is a member of LMWOA has enhanced soil reclamation specifically by minimizing soil EC and pH. These LMWOA can bind metals forming complexes and changing their bioavailability [8]. and have related to nutrient uptake, metal detoxification, and microbial communication in agricultural ecosystems [23]. [24] reported that organic acids contributed to preserving the integrity, stability, and activity of cell membranes of canola plants. Similar results have been reported about the effect of organic acids and EDTA under heavy metal stress in okra [25]

Table 2. Electrical Conductivity (EC) ds.m^{-1} in Soil

	Soil Type				
	Treatments	Soil without plant	Barley soil	Alfalfa soil	Mean
Se a w at	0	2.22	2.34	2.71	2.42
	10	7.42	4.82	3.83	5.35
	20	13.13	6.93	7,35	10.03
	30	18.70	11.33	12.57	14.20
Malic acid (mM.l^{-1}) + Seawater	2+10	9.43	3.98	3.33	5.58
	2+20	17.73	4.41	3.27	8.47
	2+30	24.54	6.84	5.27	12.21
	4+10	10.20	6.23	7.36	7.93
	4+20	19.45	6.80	8.84	11.69
	4+30	26.60	8.56	8.50	14.55
	6+10	12.27	8.88	9.18	10.11
	6+20	22.76	9,39	10.84	16.80
	6+30	28.60	11.68	12.54	17.60
	Mean	16.38	6.90	7.35	
RLSD	interaction: 1.38	treatment: 0.80	soil :0.37		

Table 3. Total organic Carbon (TOC) in Soil (%)

	Soil Type				
	Treatments	Soil without plant	Barley soil	Alfalfa soil	Mean
Seawater %	0	2.74	3.42	4.77	3.64
	10	2.82	3.66	4.56	3.64
	20	2.57	3.47	4.56	3.53
	30	2.28	3.33	4.16	3.25
Malic acid (mM.l^{-1}) + Seawater	2+10	2.99	4.20	4.76	3.98
	2+20	2.38	4.37	4.52	3.75
	2+30	2.54	4.25	4.76	3.85
	4+10	2.32	3.62	4.04	3.32
	4+20	2.41	3.47	3.32	3.06
	4+30	2.07	3.25	3.83	3.05
	6+10	1.33	2.18	2.92	2.14
	6+20	1.88	2.28	2.76	2.30
	6+30	1.77	2.96	2.94	2.55
	Mean	2.31	3.42	3.98	
	RLSD	interaction :0.12	treatments :0.07	soil :0.03	

Table 4. soil pH

		Soil Type			
	Treatments	Soil without plant	Barley soil	Alfalfa soil	Mean
Seawater %	0	9.46	8.96	8.15	8.85
	10	9.02	9.04	9.62	9.22
	20	9.39	9.34	9.81	9.51
	30	9.25	9.76	9.30	9.43
Malic acid (mM.l^{-1}) + Seawater	2+10	8.94	8.03	8.24	8.40
	2+20	8.21	8.67	8.14	8.34
	2+30	7.67	8.16	8.76	8.19
	4+10	8.50	8.87	8.28	8.55
	4+20	8.43	8.65	8.22	8.43
	4+30	8,82	8.77	8.35	8.56
	6+10	8.90	9.01	8.70	8.87
	6+20	9.14	9.06	8.69	8.96
	6+30	9.43	9.12	8.56	9.03
	Mean	8.86	8.88	8.67	
RLSD:	interaction: 1.13	treatment 0.63	soil :0.28		

Table 5. soil potassium ion (µg.ml^{-1})

		Soil Type			
	Treatments	Soil without plant	Barley soil	Alfalfa soil	Mean
Seawater %	0	1.54	1.01	1.01	1.18
	10	2.36	0.93	0.92	1.40
	20	2.45	0.74	0.74	1.31
	30	3.44	0.83	0.81	1.69
Malic acid(mM.l^{-1}) +	2+10	4.60	0.93	0.97	2.16
	2+20	4.21	0.91	0.91	2.01
	2+30	4.43	0.93	0.91	2.09
	4+10	4.46	0.96	0.99	2.13
	4+20	4.14	0.91	0.87	1.97
	4+30	4.78	0.84	0.84	2.15
	6+10	5.34	0.55	0.54	2.14
	6+20	5.57	0.76	0.84	2.39
	6+30	5.68	0.74	0.76	2.39
	Mean	4.07	0.84	0.85	
RLSD	interaction: 0.12	treatment 0.07	soil :0.03		

Table 7. soil chloride ion (µg. ml^{-1})

		Soil Type			
	Treatments	Soil without plant	Barley soil	Alfalfa soil	Mean
Seawater %	0	84.14	84.14	84.14	84.14
	10	155.26	115.78	94.39	121.81
	20	183.36	133.80	111.26	142.80
	30	208.16	156.09	136.00	166.75
Malic acid (mM.l^{-1}) + Seawater	2+10	166.50	103.67	107.60	125.92
	2+20	178.06	115.61	120.55	138.07
	2+30	199.33	119.45	124.74	147.84
	4+10	176.43	111.96	108.38	132.25
	4+20	181.00	120.37	109.92	137.09
	4+30	205.26	118.79	121.57	148.54
	6+10	211.36	127.42	129.71	156.16
	6+20	232.00	125.40	122.95	160.11
	6+30	266.73	133.18	141.85	180.58
	Mean	188.27	120.43	116.38	
RLSD	interaction17.68	treatment 10.68	soil :4.9		

4. Conclusions

We found that both barley and alfalfa plants are suitable for phytoremediation of salt affected soils. Using alfalfa is cost effective although it is a moderately saline-tolerant legume compared with salt tolerant barley plant, because it is an important forage source for livestock industries around the world, wide adaptability, high yield, and resistance to frequent cuttings. Malic acid plays an important role in enhancing phytoremediation processes, so it can be used especially at 2 mM l^{-1}. More studies are needed to engineer the rhizosphere specially the activity of microorganisms.

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Declaration of competing interest

The authors declare that they have no competing financial interest or personal relationship that could have appeared to influence the work reported in this paper.

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دور حامض الماليك في تحسين كفاءة نباتي الشعير والجت في المعالجة النباتية للتربة المتأثرة بالملوحة

سارة فيصل راضي، لمى حسين عبد القادر

قسم علوم الحياة، كلية التربية للعلوم الصرفة، جامعة البصرة، البصرة، العراق.

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الملخص

تُمثل الملوحة مشكلة عالمية متنامية ذات تأثير واضح في نمو وانتاجية المحاصيل. وقد ركزت أغلب جهود استصلاح التربة الملحية على وسائل مثل البزل السطحي أو استعمال المواد الكيميائية ذات الكلفة المرتفعة من أجل تحسين إنتاجية هذه الترب. وتشكل المعالجة بأستعمال النباتات وسيلة غير مكلفة وغير مضرة بالبيئة.

أُجريت تجربة مختبرية لدراسة دور حامض الماليك وهو من الاحماض العضوية منخفضة الوزن الجزيئي في زيادة كفاءة نباتي الشعير والجت على المعالجة النباتية للترب المتأثرة بالملوحة. تم استزراع بذور الشعير والجت في سنادين وسُقيت بمحلول هوگلاند المغذي مع ثلاث تراكيز من ماء البحر 30%, 20%, 10% وخليط من ماء البحر بالتراكيز السابقة مع حامض الماليك بالتراكيز 6, 4, 2 مليمول. لتر $^{-1}$ بالإضافة الى معاملة السيرة بمحلول هوگلاند. بعد مضي 12 أسبوعًا تم حصد النباتات واخذت ثلاث نماذج من الترب (تربة الشعير وتربة الجت والتربة بدون النبات واخضعت لتحليل صفاتها الكيميائية والفيزيائية (التوصيلية الكهربائية EC والكاربون العضوي الكلي TOC والرقم الهيدروجيني pH وأيونات الصوديوم والبوتاسيوم والكلور. بينت النتائج تسجيل خفضٍ معنوي في قيمية التوصيلية الكهربائية والرقم الهيدروجيني والبوتاسيوم والصوديوم مقابل زيادة معنوية في الكاربون العضوي الكلي لتربة الشعير وتربة الجت مقارنة مع التربة بدون نبات. وان تداخل ماء البحر مع حامض الماليك عند التركيز 2+10% سجلت زيادة معنوية في الجاهزية الحيوية للأيونات وحسنت بصورة معنوية من كفاءة النباتين في المعالجة النباتية مقارنة مع التربة بدون نبات.

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[*] **Corresponding author email :** luma.abdulqadir@uobasrah.edu.iq

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